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Randomized controlled trial

A randomized-controlled clinical trial of high fructose diets from either *Robinia* honey or free fructose and glucose in healthy normal weight males☆,☆☆

Camille Despland [a], Barbara Walther [b], Christina Kast [b], Vanessa Campos [a], Valentine Rey [a], Nathalie Stefanoni [a], Luc Tappy [a, *]

[a] Department of Physiology, University of Lausanne, Rue du Bugnon 7, CH-1005 Lausanne, Switzerland
[b] Agroscope, Institute for Food Sciences and Institute for Lifestock Sciences, Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

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SUMMARY

Background: A high fructose diet causes hypertriglyceridemia and hepatic insulin resistance in humans, but it remains unknown whether refined sugars exert different effects than naturally occurring sugars present in honey.
Aim: To assess the metabolic effects of replacing 25% total energy from starch with honey or a pure fructose:glucose mixture.
Methods: Eight healthy male were studied while consuming a controlled weight-maintenance, low fructose diet (control, CTRL), or a isocaloric diet in which 25% complex carbohydrates were substituted with either honey (HON; fructose:glucose ratio = 1.7) or fructose:glucose mixture (FG) for eight days. On day 7, participants ingested a breakfast at 7:30 am, and a lunch at 12:00 pm, both corresponding to their ongoing experimental diet. On day 8, they had an oral glucose tolerance test (OGT) with dual glucose isotopes (oral ^{13}C-, iv 6,6 $^{2}H_2$-glucose).
Results: On day 7, their postprandial glucose and insulin concentrations were lower with HON and FG than with CTRL but their plasma triglyceride concentrations and net substrate oxidation showed no difference. On day 8, there was no significant difference in glucose tolerance or postprandial suppression of glucose production between HON, FG and CTRL.
Conclusions: Consuming a diet containing 25% energy as honey or pure fructose–glucose slightly decreases postprandial blood glucose, but does not significantly increase postprandial plasma triglyceride, nor impair hepatic insulin sensitivity compared to an isocaloric starch diet.

1. Introduction

Epidemiological and experimental studies suggest that consumption of fructose-containing caloric sweeteners may cause hypertriglyceridemia, hepatic steatosis, and impaired glucose homeostasis [1]. This association may be mainly accounted for by increased total energy intake and body adiposity secondary to consumption of energy-dense, highly palatable sugary foods and beverages [2]. It has further been proposed that the fructose component of sugar may alter glucose and lipid homeostasis independently of energy balance by stimulating hepatic gluconeogenesis and de novo lipogenesis [3]. Whether this effect is due to fructose per se or to excess total energy, and whether it is also observed when fructose is consumed together with glucose remain unknown.

The most recent dietary recommendations specifically target "free" [4], "added" [5], or "extrinsic" sugars [6] to account for the fact that consumption of vegetables and fruits is inversely associated with the incidence of cardiovascular and metabolic diseases

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* Corresponding author. Tel.: +41 21 692 5541; fax: +41 21 692 5595.
E-mail addresses: camille.despland@unil.ch (C. Despland), barbara.walther@agroscope.admin.ch (B. Walther), christina.kast@agroscope.admin.ch (C. Kast), vanessacaroline.campos@unil.ch (V. Campos), valentine.rey@unil.ch (V. Rey), nathalie.stefanoni@unil.ch (N. Stefanoni), luc.tappy@unil.ch (L. Tappy).

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[7–9]. This may be related to the physical structure and dietary fiber content of fresh fruits, leading to slower gastric emptying and delayed gut absorption of monosaccharides. In addition, fruits contain a variety of micronutrients, amongst which flavonoids, polyphenols and vitamins which may prevent oxidative damages and protect against adverse metabolic effects [10,11].

Honey is classified as "added" sugar, yet is a natural product rich in polyphenols. It primarily consists of sugars (60.5–82.5%) and water (usually between 15% and 20%). Its main monosaccharide components are glucose and fructose, but the fructose:glucose ratio varies depending on its botanical origin. *Robinia* honey (often wrongly labelled as *Acacia* honey) contains more fructose than glucose. In addition honey contains disaccharides, such as sucrose, and tri- or higher oligosaccharides at lower concentrations.

Whether honey consumption exerts different health effects than other sugars remains largely unknown. On one hand, due to its low glucose content, it decreases postprandial glycemia and insulinemia, and hence may provide some advantage in people with impaired glucose tolerance [12]. On the other hand, due to its high fructose content, one may fear that it stimulates de novo lipogenesis and increases more plasma TG than sucrose. In addition, the micronutrients (polyphenols, antioxidants and rare sugars) present in honey may exert health effects independent of sugars [13].

We therefore performed a randomized, open-label, cross-over controlled trial in healthy male subjects to assess the metabolic effects of a weight-maintenance diet in which either honey from the floral nectar of *Robinia pseudoacacia* or pure fructose–glucose corresponding to 25% total energy requirements were substituted for isocaloric amounts of starch. After 6 and 7 days of each dietary condition, participants had their metabolism monitored after ingestion of breakfast and lunch, and their glucose tolerance and postprandial suppression of hepatic glucose production measured with an oral glucose tolerance test and dual ^{13}C- and ^{2}H-labelled glucose.

2. Methods

2.1. Subjects inclusion

8 male volunteers (mean age, mean weight, mean BMI) were recruited to take part to this study. Participants were eligible if they were in good health, as estimated from a medical history and standard medical examination at inclusion, nonsmokers, and did not consume more than 10 g alcohol/day. The experimental protocol was approved by the" Commission d'éthique pour la recherche humaine de l'Etat de Vaud", Switzerland (protocol 154/12, accepted on 28th of June 2012), and all participants provided written informed consent. It was performed at the Clinical Research Center of the Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland between January 2014 and March 2015. Sample size was calculated based on a power analysis to detect postprandial increases in plasma triglyceride concentration > 15%. The experimental protocol was registered at clinicaltrial.gov (NCT02747446).

2.2. Study design

Participants were studied on three different occasions according to a randomized cross-over design. On each occasion, they received during 6 consecutive days one of three different diets:

- a control, low sugar, weight maintenance diet (energy content equal to basal energy requirements calculated with the Harris–Benedict equation [14] times a physical activity factor of 1.6) containing 55% carbohydrate (50% starch and 5% sugar), 30% fat, and 15% protein (CTRL)
- a high honey, weight-maintenance diet, corresponding to diet CTRL in which 25% starch was replaced by *Robinia* honey (HON). The *Robinia* honey was produced in the year 2011 in the Southern part of Switzerland and corresponded to the sensory and melissopalynological requirements of a monofloral *Robina* honey [15]. Its sugar content, determined by HPLC [16] was, per 100 g: 40.9 g fructose, 23.9 glucose, 2.5 g maltose, 2.4 g turanose, 2.1 g erlose, 0.8 g trehalose, 0.6 g sucrose, 0.5 g isomaltose
- a high fructose + glucose, weight-maintenance diet, corresponding to CTRL diet in which 25% starch was replaced by 15.7% fructose and 9.3% glucose (FG, with a 1.7:1 fructose:glucose ratio, i.e. identical with HON).

The order with which the three diets were administered (CTRL-HON-FG, CTRL-FG-HON, HON-CTRL-FG, FG-CTRL-HON) was randomized prior to starting the experiments.

On each occasion, participants consumed the diets during six consecutive days (day 1–6) on an out-patient basis. During this period, they received all their foods and beverages under the form of pre-packed food items, with specific instructions not to consume any other food. On day 7 and on day 8, they reported at the Clinical Research Center of Lausanne University Hospital at 7:00 am after an overnight fast (Fig. 1). On arrival, subjects were asked to void their bladder, the collected urine was discarded, and all urine was thereafter collected until the end of the test for the determination of the urinary urea nitrogen excretion rate. They were also weighed, and their body composition was assessed by bio-electrical impedance analysis (Imp Df 50; ImpediMed, Pinkenba, Australia). They were then transferred to a bed, and one venous catheter was inserted into an antecubital vein of one arm and was used for blood sampling. For the second metabolic test only, a second catheter was inserted into an antecubital vein of the other arm and a primed-continuous infusion of tracer amounts of 6,6-$^{2}H_2$-glucose (Cambridge Isotope Laboratories, Cambridge, MA, U.S.; bolus 2.8 mg/kg, continuous infusion 40 μg/kg/min) was administered through this catheter throughout the metabolic test to calculate whole body glucose rates of appearance (GRa) and of disappearance (GRd). Total carbon dioxide production (VCO_2) was monitored throughout the experiment by open circuit indirect calorimetry (Quark RMR, version 9.1b, Cosmed, Roma, Italy). Heart rate of blood pressure were measured every 60 min throughout the test.

On day 7, subjects received a breakfast at 7:30 am (time 0 min), and a lunch at 12:00 pm (time 270 min) corresponding to their ongoing experimental diet (i.e. CTRL, HON or FG). Breakfast contained 25% and lunch 35% of their daily energy requirements, calculated with a physical activity factor of 1.3 to account for the absence of physical activity during these experiments. Blood samples were collected in fasting conditions (time 0 and 30 min) and every 60 min after ingestion of two meal test (breakfast at 30 min and lunch at 270 min) until time 570 min.

On day 8, participant's glucose tolerance was assessed with a dual ^{2}H–^{13}C isotope oral glucose tolerance test (OGTT). Blood samples were collected immediately before starting the 6,6-$^{2}H_2$-glucose administration ($T = -120$ min) and after 95 min tracer equilibration (−15 min). Thereafter, they ingested a solution containing 75 g glucose and 37.5 mg $^{13}C_6$ glucose (final glucose ^{13}C enrichment = 0.5% MPE) over a 5-min period, and blood samples were at 15, 30, 60, 90, 120, 180 and 240 min spent in fasting conditions. Hormones and metabolites concentrations and plasma 6,6-$^{2}H_2$-glucose were measured on each blood samples. Breath samples were collected for the measurement of $^{13}CO_2$ isotopic enrichment at $T = -120$, −15, 0, 15, 30, 60, 90, 120, 180, 240.

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